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Michael O. Leavitt, Administrator
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Comments on the HPV test plan for ethenyl arylbromo derivatives of benzene (dibromostyrene)

Dear Administrator Leavitt:

The following comments on the Great Lakes Chemical Corporation's test plan for dibromostyrene (CAS no. 125904-11-2) are submitted on behalf of People for the Ethical Treatment of Animals, the Physicians Committee for Responsible Medicine, the Humane Society of the United States, the Doris Day Animal League, and Earth Island Institute. These animal, health, and environmental protection organizations have a combined membership of more than ten million Americans.

The Great Lakes Chemical Corporation plans to carry out an acute toxicity test on fish. Assuming that this is to be carried out in accordance with OECD test guideline 203, it will kill at least 120 fish.

Most importantly, this proposal clearly contravenes the EPA's recommendations. The EPA has stated that acute fish tests are inappropriate for compounds with log $K_{o/w}$ values above 4.2 (EPA, *Federal Register* 2000, p. 81695). The log $K_{o/w}$ value of dibromostyrene has been determined to be 4.43 (test plan, p. 5). The proposed tests should therefore be withdrawn.

In addition, fish tests are not intended to predict toxicity to individual fish, but to predict economic loss to commercial and "sport" fisheries, and ecologic damage. The fish test therefore aims to show whether exposure to dibromostyrene will result in large-scale fish death. Because water pollution kills the food on which fish subsist, it can deplete fish populations even without direct fish toxicity. However, the toxicity of dibromostyrene towards aquatic plants and invertebrates is currently unknown (test plan, p. 7) and fish tests are therefore premature.

Second, *in vitro* test methods are available. The recently validated *Dar*T Test (Nagel 2002) is a prospective replacement for *in vivo* tests. The test protocol and performance parameters are described in detail in Schulte (1994) and Nagel (1998). Briefly, the *Dar*T test uses fertilized zebrafish eggs as a surrogate for living fish; because the eggs will not hatch during the test period, the *Dar*T is classified as a non-animal test. The exposure period is 48 hours, and assessed endpoints include coagulation, development of blastula, gastrulation, termination of gastrulation, development of somites, movements, extension of the tail, development of eyes, heartbeat, circulation, heart rate, pigmentation, and edema. Endpoints comparable to lethality *in vivo* include failure to complete gastrulation after 12-hours, no somites after 16-hours, no heartbeat after 48-hours, and coagrulated eggs. The other endpoints provide further insight for a more

detailed assessment of the effects of test substances. The reliability and relevance of the *Dar*T test have recently been confirmed through an international, multi-laboratory validation study coordinated and financed by the German Environmental Protection Agency; and predictions of acute toxicity from the *Dar*T test were highly concordant with *in vivo* reference data (Schulte 1996). This *in vitro* test has been accepted in Germany as a replacement for the use of fish in the assessment of wastewater effluent (Friccius 1995), and has since been nominated for development into an OECD test guideline. It is clearly suitable for immediate use as a replacement for the use of fish in SIDS screening studies.

Another promising *in vitro* assay is TETRATOX. In this assay, the protozoan *Tetrahymena pyriformis* is used as a biomarker for acute lethality in fish (Schultz 1997). The biochemistry and physiology of *T. pyriformis* have been thoroughly investigated since the 1950s and this assay has been used, in various forms, for aquatic toxicity testing since the 1970s (Sinks 2001). In this test, a range-finding study followed by three replicate definitive tests is performed for each test substance. Each treatment replicate consists of a minimum of five different concentrations per substance tested; thus, at least 30 data points make up each analysis. The current, standardized protocol is for a 40-hour static test, which provides for multigenerational exposure. Range-finding tests are also included to allow an accurate approximation of both the highest concentration with no observed effect on population growth and the lowest concentration with total inhibition of cell replication. Output measures from the TETRATOX assay are the 50% inhibitory growth concentration (IGC50, mmol/L) and the 95% fiducial interval. The current TETRATOX database includes more than 2,000 industrial organic chemicals, including over 800 aliphatic chemicals, 900 aromatic chemicals, 400 neutral narcotics, and 400 direct-acting electrophiles, among others (Schultz, personal communication). The TETRATOX protocol has now been standardized and has undergone a preliminary ring test (Larsen 1997). The German EPA is currently funding a second, more elaborate ring test, with the goal of establishing an OECD test guideline. In the meantime, data generated by TETRATOX demonstrate a consistently high degree of concordance with data from *in vivo* acute studies in fish, which supports the use of this assay as a replacement for toxicity studies in fish (Seward 2001).

Thank you for considering these comments. Please feel free to contact me at 757-622-7382, ext. 8001, or via e-mail at JessicaS@peta.org.

Sincerely,

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Federal Agency Liaison

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